

- volume, wherein said measuring volume is arranged at a distance of  $\leq 1000 \mu\text{m}$  from a laser focusing optic,
- b) measuring said fluorescence at measuring times of  $\leq 500$  ms using detecting optics,
  - c) determining material-specific parameters of said molecule.

108. The method according to claim 107, wherein said measuring volume comprises  $\leq 10^{-14}$  l.


109. The method according to claim 107, wherein a concentration of said molecule or molecules to be assayed amounts to  $\leq 1 \mu\text{M}$ .

110. The method according to claim 107, wherein said material-specific parameters are translational diffusion coefficients, rotational diffusion coefficients, excitation and emission wavelengths, life of the excited state of said substituent, or combinations thereof.

111. The method according to claim 107, wherein a change of coordinates of said measuring volume with times defines an apparent diffusion time of said molecule or molecules.

112. The method according to claim 107, wherein said substituent is a chromophorous ligand, a luminophorous ligand, or a luminophore-labeled ligand having spectroscopic parameters which are correlated with a property or function of said molecule.

113. The method according to claim 110, comprising determining a translational diffusion, rotational diffusion, or both said translational and rotational diffusion, and further comprising a functional evaluation of said molecule by

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- determining the absolute number of molecules in said measuring volume,
  - determining variations, with time, of the absolute number of molecules in said measuring volume,
  - determining specific concentrations of structurally distinct ligands or ligand-molecule complexes in said measuring volume,
  - or combinations thereof

and deriving thereof

- thermodynamic binding constants between said ligands and said molecules,

- rate constants of recognition reactions between said ligands and said molecules,
- rate constants of enzymatic processes involving complexes formed between said ligands and said molecules,
- or combinations thereof.

114. The method according to claim 113, wherein said molecules or molecule-ligand complexes are ionic.

115. The method according to claim 113, wherein said molecules or molecule-ligand complexes are non-ionic.

116. The method according to claim 107, wherein measuring takes place within a superimposed electric or magnetic field, which is constant or varying with time.

117. The method according to claim 116, wherein ionic molecules or molecule-ligand complexes are forced through said measuring volume, or held in said measuring volume by a rectified electric field or an alternating electric field.

118. The method according to claim 116, wherein measuring takes place within said electric field effecting an electric molecular trap, and wherein a luminophore-labeled ligand bears a smaller charge than, or a charge opposite to that of, a target molecule which forms a complex with said ligand.

119. The method according to claim 118 further comprising electrophoretic separation of free luminophore-labeled ligands from specifically complexed ligands.


120. The method according to claim 119, wherein said free luminophore-labeled ligands are nucleic acid probes.

121. The method according to claim 119, wherein said specifically complexed ligands are nucleic acid hybrids.

122. The method according to claim 116 further comprising, prior to said exposing step, concentrating complexes of the labeled ligand and the molecule in a first electrophoresis step and transporting said complexes formed into said measuring volume in a second electrophoresis step.

123. The method according to claim 109, wherein said substituent is a luminophorous ligand having an extinction coefficient  $\geq 30,000$  with a quantum yield  $\geq 0.1$ , or said substituent is a chromophorous ligand, which comprises one or more dye oligomers.

124. The method according to claim 107, wherein said laser-focusing optics comprises an emergency objective which is either directly in contact with the sample or separated from the sample only by a transparent sheet.


 125. The method according to claim 107, involving assaying a molecule or molecules in a plurality of samples, whereby said sample volumes are arranged two-dimensionally on a membrane, sheet or wafer surface.

126. The method according to claim 107, involving assaying a molecule or molecules in a plurality of samples, whereby said sample volumes are arranged linearly in a capillary system.

127. The method according to claim 107, wherein said sample comprises natural cells, or cells modified *in vitro*, or artificially prepared vesicular structures.

128. The method according to claim 107, wherein said samples are generated by a microdispensing system.

129. The method according to claim 107, wherein an access to phenotypically selected genotypes on DNA or RNA level is made possible by the use of photochemically activatable reagents.



130. The method according to claim 107, wherein said assay screens a substance for its potential pharmacological activity by its interaction with specific receptors and wherein binding of a luminescent-labeled ligand to said receptor is a function of said interaction.


131. The method according to claim 130, wherein natural receptors on their carrier cells as well as receptors on receptor-overexpressing carrier cells, or receptors in the form of expressed molecules or molecular complexes are used.

132. The method according to claim 130, comprising at least two types of receptors, whose differential binding potential is determined through interfering binding of variants of potentially pharmacological active substances and a luminescent-labeled natural ligand.

133. The method according to claim 131, wherein the cells are capable of dividing or are metabolically active.

134. The method according to claim 130, wherein said substance is present in complex natural, synthetic, or semisynthetic mixtures, and said mixtures are subjected to chromatographic separation into samples prior to said assay.

135. The method according to claim 107, wherein a type, or number, or both the type and number of homologously complementary nucleic acid molecules in a sample are analyzed by a labeled nucleic acid probe through hybridization.



136. The method according to claim 135, wherein an excess of said labeled probes is provided and said labeled probes are single-stranded synthetic or cellular RNAs or DNAs with a particular polarity (+ or - strand).

137. The method according to claim 135, wherein the reaction rate of complex formation in hybridization is accelerated by performing the assay in a medium containing chaotropic salts, or organic solvents, or both chaotropic salts and organic solvents.

138. The method according to claim 135, wherein the degree of complementarity of the hybridized nucleic acid is analyzed through the thermodynamic stability of the complex.

139. The method according to claim 135, wherein the detection of a complementary nucleic acid is quantified by i) using an internal standard, whose sequence differs from the sequence of the nucleic acid to be quantified in at least one point mutation and ii) performing the analysis at a temperature at which the different conformations of the complexes of the probe with the internal standard and of the probe with the nucleic acid molecule to be analyzed are distinct with respect to translational diffusion, or rotational diffusion, or both the translational diffusion and rotational diffusion.


140. The method according to claim 107, assaying two different molecules together in one sample through a reaction of two different ligands which are labeled with different dyes, wherein the dyes are either excited with light of different wavelengths or independently detected by light of different emission wavelengths.

141. The method according to claim 107, wherein the molecule is complexed simultaneously with two ligands which are each labeled with optically



distinct fluorescent dyes and the simultaneous complex formation is detected either through

- formation of an energy transfer complex,
- correlation in time of the signals having different wavelengths of excitation,
- correlation in time of the signals having different wavelengths of emission,
- or combinations thereof.


 142. The method according to claim 107, wherein said samples are analyzed for mixtures of vesicular structures by

- staining the vesicles with fluorescent-dye labeled antibodies, or
- incorporating luminophore-labeled ligands specifically and permanently into the vesicular structure,
- or a combination thereof.

143. The method according to claim 142, wherein said vesicles bear lipids of the VLDL, LDL or HDL types.

144. The method according to claim 107, wherein the products of an *in vitro* protein biosynthesis are analyzed for specific binding properties or enzymatic properties.

145. The method according to claim 107, wherein mixtures of different oligomers or polymers are analyzed for average translational diffusion coefficients, or average rotational diffusion coefficients, or both average translational diffusion coefficients and average rotational diffusion coefficients.

 146. The method according to claim 107, comprising a three-dimensionally compartmentalized sample to be assayed, wherein the dynamics or reaction kinetics of particular molecules in a plurality of measuring volumes as well as the positional coordinates of said volumes are registered in order to assemble a two- or three-dimensional image thereof.

147. The method according to claim 107, assaying samples for complex formation between unlabeled ligands and molecules in competition with luminophore-labeled ligands wherein assaying is performed i) in solution, ii) by coupling of molecules to a solid phase or iii) by using cell-associated molecules.--